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The curvature of *Physcomitrium patens* caulonemal filaments in relation to their capacity for phototropic and thigmotropic responses and nutation

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Abstract
Light-grown, whole gametophytic colonies of *Physcomitrium patens* exhibit a spiral morphology resulting from the strongly co-ordinated curvature of the population of peripheral caulonemal filaments. The direction of curvature is predominantly clockwise when cultures are illuminated from above and anticlockwise when illuminated from below. Transferring cultures from top to bottom illumination provided a means of estimating the cell cycle time for caulonemal apical cells. In *Physcomitrium*, side branch initials emerge from caulonemal subapical cells on the outside of the curve. By contrast, the curvature of the caulonemata of *Funaria hygrometrica* is predominantly anticlockwise when colonies are illuminated from above and clockwise when illuminated from below. In *Funaria*, side branch initials emerge from subapical caulonemal cells on the inside of the curve. We have discounted a role for gravity in these phenomena and discuss possible mechanistic explanations in terms of aberrant nutation and phototropic or thigmotropic responses on the slippery, solid agar medium. We describe the first known case of thigmotropism of protonemata of a bryophyte. Thigmotropism occurs in response to crowding of protonemal filaments in a thin layer of agar medium and is restricted to caulonemata.

Keywords: Caulonemata, curvature, nutation, phototropism, thigmotropism

Introduction
Preliminary observations had shown that caulonemal filaments of *Physcomitrium patens* exhibit predominantly clockwise curvature on the surface of solid growth medium when illuminated from above. Computer modelling suggested that this is important for the generation of a normal morphology, including the establishment of circular symmetry by protonemal colonies (Fracchia and Ashton 1995). Here we examine the curvature of *Physcomitrium* caulonemata in relation to their capacity for phototropic and thigmotropic responses and nutation.

Material and Methods
The *Physcomitrium* line used in this study was derived from a subculture of Gransden 1962 *Physcomitrium* (formerly *Physcomitrella*) *patens* obtained from a single spore isolated from nature (Ashton and Cove 1977). *NicB5ylo6* was obtained by *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine mutagenesis of the wild-type *Physcomitrium* (Ashton and Cove 1977). The *Funaria* line was derived from a single *Funaria hygrometrica* spore.
Plants were grown axenically on solid ABC medium (Knight et al. 1988) supplemented with nicotinic acid (8 µM), with or without di-ammonium (+) tartrate (5 mM) at approx. 22-25 °C for 3 to 5 weeks. In some cases, the medium was overlaid with sterile, porous cellophane discs (Grimsley et al. 1977), in turn covered with a thin layer of agar medium (1-2 mm deep). Continuous white light culture conditions, described previously (Ashton et al. 1985), were utilised with the following modifications.

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1. Petri dishes were covered with one layer of clear resin filter (Roscolux No. 114, Hamburg Frost) and illuminated from above, i.e. through the lid of the dish. 2. Petri dishes were inverted and covered with a layer of clear resin filter and illuminated from above, i.e. through the bottom of the dish. 3. Petri dishes were placed on a raised pane of clear glass and illuminated from below, i.e. through the bottom of the dish. 4. Petri dishes were initially illuminated from above (as described in 1) for 18 d and subsequently moved and illuminated from below (as described in 3) for 9 d. This last treatment facilitated estimating the cell cycle time of apical caulonemal cells.
Averages and sample standard deviations were calculated using Microsoft Excel.

Results and Discussion

Caulonemal filaments of *Physcomitrium*, when illuminated from above, grew over the surface of solid agar medium perpendicular to the incidence of light or slightly negatively phototropically into the medium. They curved at a rate of approximately 1° per apical cell cycle (Table 1), which had a duration of approximately 10 h (Table 2).

Table 1. Rate of curvature in degrees of *Physcomitrium* caulonemal apical cells.

Filament number	Number of cells per caulonemal filament	[1]Curvature of filament in degrees	Curvature in degrees per cell
1	71	82	1.2
2	64	60	0.94
3	57	70	1.2
4	68	60	0.88
5	78	68	0.87
[2]Ave	68	68	1.0
[3]StDev.S	7.8	9.1	0.17

[1]Angle in degrees between a line through the central long axis of the caulonemal basal cell and a line through the central long axis of the caulonemal apical cell.
[2]Ave = average of values in each column (n = 5).
[3]StDev.S = Sample standard deviation of values in each column.
Values are given to two significant figures.

Table 2. Caulonemal apical cell cycle time.

Caulonemal filament	[1]Number of new cells	[2]Growth period (h)	Cell cycle time
1	23	216.5	9.4
2	21	217	10
[3]Ave	22		9.9
[4]StDev.S	1.4		0.65

[1]Number of new cells refers to cells formed after transferring colonies from illumination from above to illumination from below, which resulted in a reversal of the direction of curvature.
[2]The number of hours grown in illumination from below.
[3]Ave = average of values in each column (n = 2).
[4]StDev.S = Sample standard deviation.
Values in columns 2 and 4 are given to two significant figures.

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Evidence was also obtained that, as in the roots of *Arabidopsis* seedlings (Okada & Shimura, 1990), curvature may be the result of an aberrant tropic response on the slippery agar surface. However, it is unclear whether the tropic response concerned is thigmotropic or phototropic. In support of a thigmotropic mechanism was the discovery that, when caulonemal filaments were grown in a thin layer of agar medium, the crowded filaments contacted each other and wound around one another producing a multi-stranded, rope-like structure (Figure 1A and B).

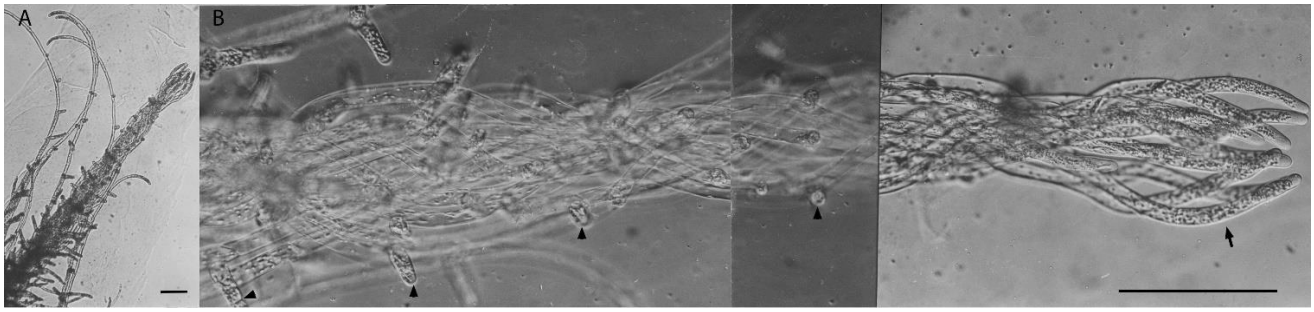

Fig. 1A. A caulonemal rope comprised of multiple, intertwined caulonemata and four individual, curved caulonemata with side branch initials and secondary chloronemata. **B.** Enlarged, composite image of a caulonemal rope. Arrow points to caulonemal apical cells; arrowheads indicate side branch initials and developing secondary chloronemata. Scale bars represent 150 µm.

It has been reported that rhizoids of some moss species, e.g. *Fontinalis squamosa* (Glime 1987) and *Calliergon stramineum* (Duckett 1994), are thigmotropic and coil tightly around any solid object (Goode et al. 1992). The main axes of rhizoids frequently wind around each other and fine ramifications of rhizoids wind around larger rhizoids forming 'rhizoid wicks' (Duckett et al. 1998). These are especially well developed in the Polytrichales (Wigglesworth 1947) and resemble quite closely the 'caulonemal ropes' we describe above. However, our report is of the first documented case of thigmotropism of protonemata of *Physcomitrium* and appears to be restricted to caulonemal filaments since secondary chloronemal filaments arising from caulonemata did not display this response. Furthermore, when caulonemal apical cells were caused experimentally, by irrigating with sterile distilled water, to dedifferentiate into chloronemal apical cells, the filaments derived from them grew apart, producing a structure reminiscent of a cut rope with the individual strands at the cut end splayed apart.

Evidence in support of a phototropic mechanism was obtained by growing cultures with illumination arriving through the Petri dish lid (see condition 1 in Materials and Methods), through the bottom of the Petri dish (see conditions 2 and 3) or initially from above and then from below (see condition 4). The direction of curvature of the caulonemata depended on the direction of the incident light (Table 3). In condition 1 (light from above), the curvature was predominantly clockwise; in conditions 2 and 3 (dish inverted or illuminated from below), it was predominantly anticlockwise, while in condition 4 after illuminating for a period with the light coming from above and then illuminating from below, the direction of curvature changed from clockwise to anticlockwise. These findings suggest that, as a mechanistic explanation of filament curvature, the occurrence of an aberrant phototropic response on the surface of slippery agar medium is equally as plausible as an aberrant thigmotropic response.

It was noted also that curved caulonemata possessed oblique dividing cross walls whereas chloronemata lacking curvature had perpendicular cross walls. Although we do not know whether the occurrence of oblique cross walls plays a causative role in filament curvature or is a consequence of it or is unrelated to it, it is plausible that curvature of the apical cell generates asymmetrical tensions in the cytoskeleton that in turn could be responsible for repositioning the mitotic spindle and cell plate during division resulting in the formation of an oblique cross wall. Evidence supporting this was obtained by Bopp and Brandes (1969), who used time-lapse photography to demonstrate that the

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spindle and cell plate were re-orientated respectively from being in line with or perpendicular to the central long axis of the apical caulonemal cell to a position corresponding to the oblique cross wall subsequently laid down.

Table 3. Direction of curvature of *Physcomitrium* caulonemata.

Physcomitrium grown on nitrate	Illuminated through Petri dish lid			Illuminated through bottom of Petri dish		
	Anticlockwise	Straight	Clockwise	Anticlockwise	Straight	Clockwise
Ave[1]	13	17	70	63	22	14
StDev.S[2]	14	13	17	7.7	6.8	8.8
n[3]	26			8		
Physcomitrium on ammonium	Illuminated through Petri dish lid			Illuminated through bottom of Petri dish		
	Anticlockwise	Straight	Clockwise	Anticlockwise	Straight	Clockwise
Ave	0.00	20	80	62	32	5.0
StDev.S	0.00	17	17	0.00	0.00	0.00
n	5			2		
Funaria on nitrate	Illuminated through Petri dish lid					
	Anticlockwise	Straight	Clockwise			
Ave	93	4.5	2.4			
StDev.S	6.0	3.6	3.3			
n	5					

[1]Ave = average of percentages of caulonemal filaments/colony exhibiting anticlockwise, straight or clockwise curvature for n colonies.
[2]StDev.S = Sample standard deviation of scores for n colonies.
[3]n = number of colonies.
Values are given to two significant figures.

Also, of interest regarding the curvature of caulonemata was the sidedness of emergence of side branch initials (SBIs). SBIs emerged from the second or third subapical cell of caulonemata at the distal end of the cell and next to the acute angle generated by the oblique cross wall. In *Physcomitrium*, the SBIs always emerged on the outside of the curve and developed into positively phototropic, branching secondary chloronemata (85-90%) or into secondary caulonemata (5-6%) or gametophores (Ashton and Cove 1990, Figure 2A and B). Contrastingly, when illuminated from above, the caulonemata of *Funaria hygrometrica* exhibited predominantly anticlockwise curvature with secondary protonemal branches emerging on the inside of the curve (Table 3, Figure 2C). This confirms the qualitative observation by Bopp (1959), who also noted that, when illuminated from below, the curvature of caulonemata changed to clockwise. Currently, we don't have an explanation for these differences between two closely related members of the Funariaceae.

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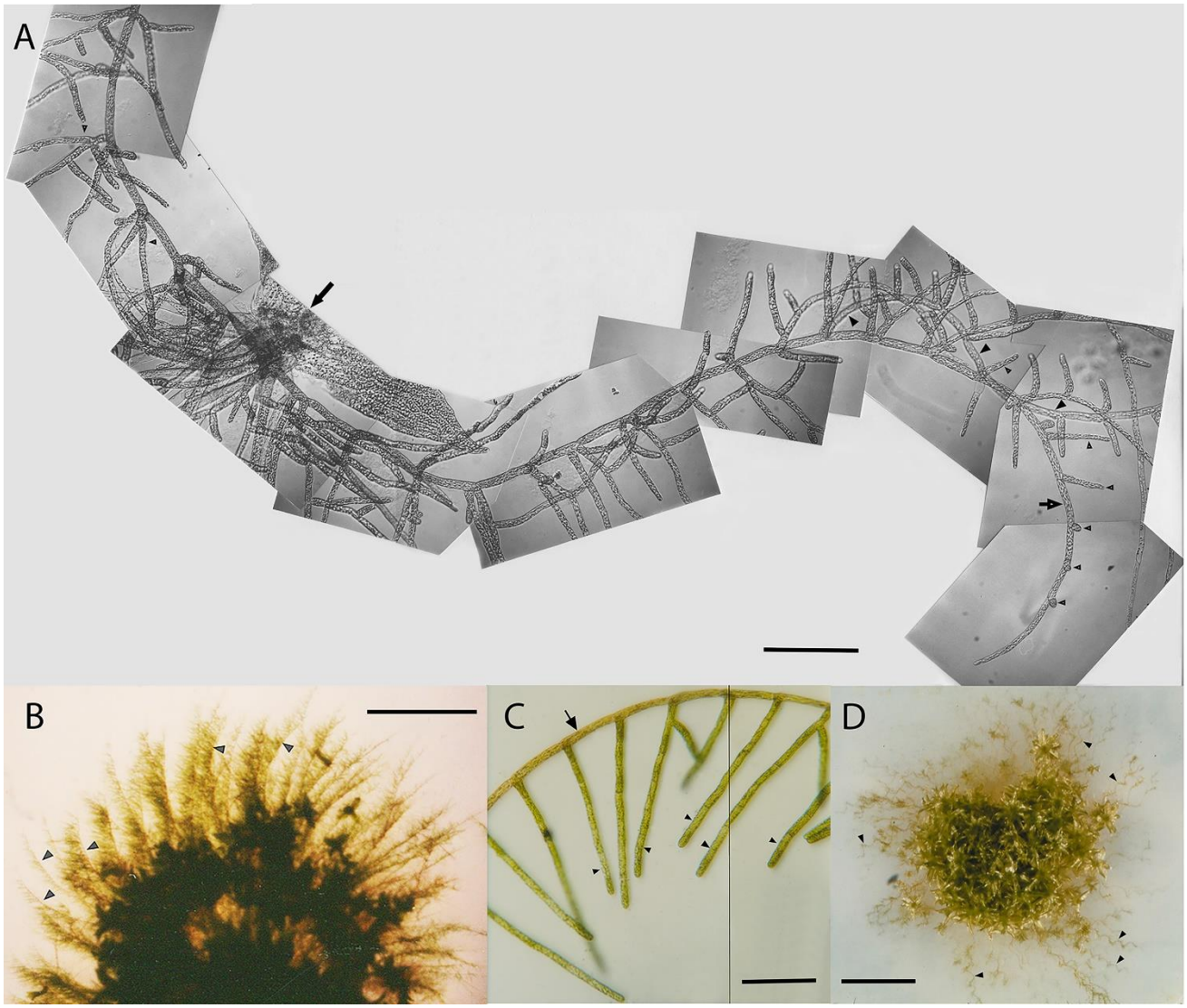

Fig. 2A. Composite image of an individual caulonema of *Physcomitrium*. Short arrow points to a curved, primary caulonemal filament. Small arrowheads indicate side branch initials, simple secondary chloronemal filaments and compound chloronemata emerging on the outside of the curves of the primary caulonema. Large arrowheads indicate a secondary caulonema, also emerging on the outside of the curve. Long arrow points to the base of a leafy gametophore with rhizoids. **B.** Half of a gametophytic colony of *Physcomitrium* with spiral morphology. Arrowheads indicate peripheral caulonemata with clockwise curvature and secondary chloronemata emerging on the outside of the curve. **C.** Composite image of part of an individual caulonema of *Funaria*. Arrow points to a primary caulonema with anticlockwise curvature. Arrowheads indicate secondary chloronemata emerging on the inside of the curve. **D.** A gametophytic colony of *Physcomitrium nicB5ylo6*. Arrowheads point to wavy, peripheral primary caulonemata. All cultures were illuminated in continuous white light from above. Scale bars represent 300 µm (**A**, **C)** and 5 mm (**B**, **D**).

Clockwise curvature of *Ceratodon purpureus* caulonemata has been observed in space under conditions of microgravity and the absence of light by Kern et al. (2005), who consequently proposed that the ability of caulonemal apical cells to curve is an innate property. A possible mechanistic explanation is that the caulonemal apical cell nutates in a circular or elliptical manner and slides on the surface of the slippery, solid agar medium. The direction of nutation, clockwise or anticlockwise, would then determine the direction in which the apical cell slides and thus the direction in which it curves. Assuming this is the same phenomenon we have observed in the light, we can conclude that

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the direction of nutation is influenced by the direction of the incident light. We can also infer that nutation does not involve rotation around the long axis of the caulonemal apical cell since this would result in twisting of the apical cell and its mitotic derivatives, which in turn would be expected to lead to the emergence of SBIs on all sides of subapical caulonemal cells instead of only on the outside of the curve. Little has been written about nutation in bryophytes, but time-lapse photography appears to show small alternating changes of growth direction of elongating caulonemal apical cells of *Funaria* (Bopp and Brandes 1969). Also, we have obtained, by mutagenesis with NTG, a line of *Physcomitrium*, *nicB5ylo6*, which is partly characterised by wavy caulonemal filaments (Figure 2D). This phenotype is very similar to the wavy phenotype of young seedlings of *Arabidopsis* growing on the surface of solid agar medium inclined at an angle of 45° to the direction of gravity (Okada and Shimura 1990) and we propose that in both cases it might be caused by repeated reversal of the direction of nutation and thus of sliding on the slippery agar medium. We also suggest that *nicB5ylo6* possesses one or more additional mutations responsible for the wavy phenotype since it seems unlikely that the *nic* or *ylo* mutation is the cause. It is interesting that at least six genes influence the wavy phenotype of *Arabidopsis* seedlings (Okada and Shimura 1990).

As is the case with caulonemata grown in space and in darkness, gravity has no role in determining the direction of curvature in light-grown cultures for the following reasons. 1. Gravitropic responses in mosses are turned off by light at high photon fluxes such as those used in our study (Knight and Cove, 1989). 2. Gravity is uniform across whole moss colonies in our experiments but, while the curvature of caulonemata was predominantly clockwise or anticlockwise depending on the direction of light, colonies were also characterised by the possession of collections of straight caulonemata or caulonemata of opposite curvature in regions of the colonies distinct from that displaying the predominant curvature (Table 3). Also, very occasionally the direction of curvature of an individual caulonemal filament was reversed (Figure 2A). We believe the most probable explanation for this is non-uniformity of the direction of light resulting from reflection from the bottom of the Petri dish. Finally, we disagree with Kern et al. (2005) that the curvature of caulonemata and the associated spiral growth pattern of whole moss colonies observed in microgravity in space is overridden by a constant gravity vector on earth, at least in *Physcomitrium* and *Funaria*.

Notes on contributors

Neil W. Ashton is Professor Emeritus of the University of Regina. He began his research on *Physcomitrella* (now *Physcomitrium*) *patens* in 1980 as a graduate student in the Genetics Department, University of Cambridge and has continued studying this model plant system to the present day.

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Disclosure statement

The authors report there are no competing interests to declare.

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